Physiological characteristics changes of *Aesculus chinensis* seeds during natural dehydration

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Abstract: A study was conducted to determine the physiological characteristics changes of *Aesculus chinensis* seeds during natural dehydration in 2003. The results showed that *A. chinensis* seeds were recalcitrant with being highly desiccation-sensitive. The seed moisture content of fresh fruits was higher than 60%. When the seeds were naturally dried for 30 days, their moisture content declined to 30.2% and their viability was completely lost. The seed germination percentage had a small increase at the beginning of desiccation and then decreased rapidly. The relative electrical conductivity of the *A. chinensis* seeds increased along with a decrease in seed moisture content. However, there was an abnormal increase in relative electrical conductivity when the seed moisture content was between 53.7% and 50.9%. Superoxide dismutase (SOD) activity decreased rapidly in the period of desiccation except for an abnormality when the seed moisture content was between 53.7% and 50.9%. Malondialdehyde (MDA) content increased slowly at the early stage of desiccation and then rose rapidly after the moisture content was below 50.9%. The soluble sugar content in seeds slowly increased with the increasing period of desiccation. The seed germination percentage was at the high level when seed moisture content was in range of 47%– 60%, which suggests that this was the optimum moisture content for maintaining *A. chinensis* seed viability.

Keywords: *Aesculus chinensis;* Seeds; Air drying; Physiological characteristics; Seed moisture content; Seed germination percentage; Superoxide dismutase (SOD) activity; Malondialdehyde (MDA) content; Seed electrical conductivity

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Introduction

The seeds of *Aesculus chinensis* belong to the recalcitrant category according to Roberts (1973). They are sensitive to desiccation but a little bit tolerant to low temperature. They can probably be stored at 0 °C because the species originates from a temperate zone in Northern China. However, the longevity of these seeds is only about half a year if they are kept moist and stored at an ambient temperature (0–5°C), (SFA General Administration of Forest Farms, Tree Seeds and Seedlings 2001). The short lifespan is a major problem for both long-term conservation of germplasm and commercial seed storage, making it important to study ways to improve seed storage potential.

The mechanism of desiccation tolerance in recalcitrant seeds is one of the key topics for seed scientists. However, specific mechanisms associated with the desiccation sensitivity of recalcitrant seeds are still not resolved. Desiccation sensitivity is a complex physiological phenomenon and is likely to be controlled by inefficiency of protective mechanisms and a series of deleterious events.

Pammenter *et al*. (1999) found that the causes of death in recalcitrant seeds after desiccation were the impairment of specific metabolic activities and probable free radical attacks, and oxidative damage. Li and Sun (1999) also reported that the desiccation sensitivity of cocoa axes and cotyledons was correlated with a rapid decrease in enzymic protection against oxidative attack. Others pointed out that the loss of viability during drying in several desiccation-sensitive seeds was accompanied by increased lipid peroxidation and the accumulation of stable free radicals (Hendry *et al*. 1992; Chaitanya *et al*. 1994; Finch-Savage *et al*. 1996). Changes of carbohydrates and proteins were also observed although different results were recorded by different scientists. Li and Sun (1999) concluded that recalcitrance of cocoa axes was not caused by a lack of sugar-related protective mechanisms, but Connor and Sowa (2003) noted that the accumulation of sucrose during desiccation of *Quercus alba* seeds may have acted secondarily as a glycoprotectant, preventing both desiccation damage of cell membranes and cell collapse. Connor and Sowa (2003) also found that the most sensitive indicator of desiccation damage was the irreversible change in protein secondary structure in embryonic axes and cotyledon tissue. After investigating ten coffee species, Chabrillange *et al*. (2000) reported that no significant relationship was found between seed desiccation sensitivity and soluble sugar content.

A. chinensis is becoming an important tree species in afforestation and reforestation in China, thus more and more *A. chinensis* seeds are being needed for forest nurseries. Little is known, however, about the physiological characteristics of these seeds. The effect of desiccation on the physiological changes of *A. chinensis* seeds was investigated in this study.

Materials and methods

The seeds of *A. chinensis* were collected from Hanzhong, Shaanxi Province in the middle of October 2003 after they had been shed. The seeds were kept moist immediately after collection and shipped to Nanjing Forestry University in two days by using big cane-baskets with sawdust. The seeds for experiments were manually chosen for similarity in size.

The seed desiccation experiment was carried out on a laboratory table at a room temperature of 10–20°C and relative humidity of 75%±5%. Samples were randomly taken for germination testing and enzyme activity determination every three days. The

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desiccation experiment lasted 30 days and 11 samples were taken.

Moisture content analyses

Seed moisture content was determined according to the criteria of the International Rules for Seed Testing (ISTA 1996). The sample seeds were pre-dried at 70°C for five hours and then sliced into small pieces and dried at (103±2) °C for 17 h. Moisture content was expressed as a percentage of fresh weight.

Germination test

Seed germination testing was performed according to criteria of the International Rules for Seed Testing (ISTA 1996) with 4×25 seeds being sampled for each test. The only pretreatment was conducted at a 60-second submersion in a 0.1% solution of sodium hypochlorite. After being rinsed in running tap water, seeds were placed on sterilized fine sand and incubated in a growth chamber at a constant temperature of 25 °C for one month. A seed was scored as germinated when both radicle and plumule appeared without obvious abnormalities.

Electrical conductivity test

Fifteen sound seeds with no apparent injury in uniform size were sampled for each electrical conductivity test. The seeds were washed by using running tap water, rinsed by using double distilled water, and then water on the seed coat was finally absorbed by using filter paper. The treated seeds were soaked in a beaker in 150 mL-double-distilled water at 25°C for 24 h, and a initial electrical conductivity (S_1) was measured by using a conductivity meter (Model DDS-307 made in China). After that, the beaker mouth was sealed by using plastic film, and the solution was then boiled for 15 min to kill the seeds. After 30-min cooling, the final electrical conductivity (S_2) was measured by using double distilled water as a reference. The relative electrical conductivity was calculated by using the following equation:

$$L = \frac{S_1 - E}{S_2 - \mathrm{E}} \tag{1}$$

where, L is the Relativeelectrical cinductivity, S_1 the initial electrical conductivity, S_2 the final electrical conductivity, and E the electrical conductivity of control.

Determination of SOD activity and MDA content

The SOD activity and MDA content of each desiccated seed sample was immediately tested by using the method described by Zhao (1995).

Determination of soluble sugar content

The soluble sugar content of each desiccated seed sample was determined by using an anthrone colorimetric method described by Zhao (1995).

Statistical analysis

The data was analyzed by using SAS6.12 and EXCEL software.

Results

Effect of desiccation on seed moisture content and germination percentage

The moisture content of *A. chinensis* seeds reduced gradually during air drying (Fig. 1). The seed germination percentage increased slightly at the beginning (from 79% to 85%) and then decreased rapidly (Fig. 2), indicating slight desiccation had some beneficial effects on seed germination at the early stage of water loss. However, the seed germination percentage decreased rapidly after 12 days of desiccation when the seed moisture content reduced to 47.3%. After 30 days, the germination percentage declined to zero when seed moisture content had decreased to 30.2%.

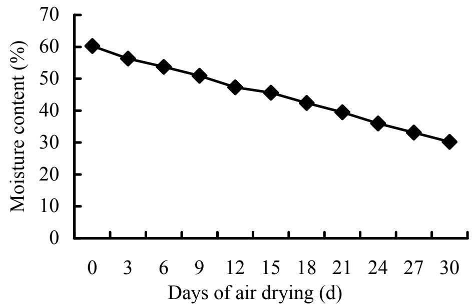

Fig. 1 Effect of desiccation on seed moisture content

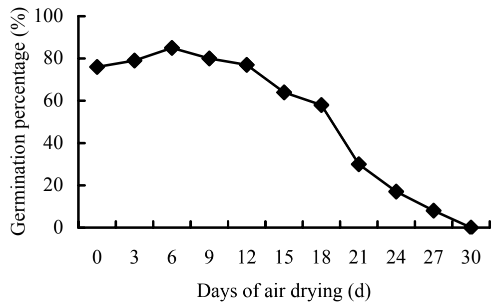

Fig. 2 Effect of air drying on germination percentage of *Aesculus chinensis* seeds

Effect of desiccation on seed electrical conductivity

Fig. 3 showed that seed electrical conductivity increased with the decrease of seed moisture content. However, there was an abnormal increase when the moisture content was between 53.7% and 50.9%. One possible explanation was that the cell membrane was more easily injured at the beginning of desiccation, but could recover to some extent after desiccating adaptation.

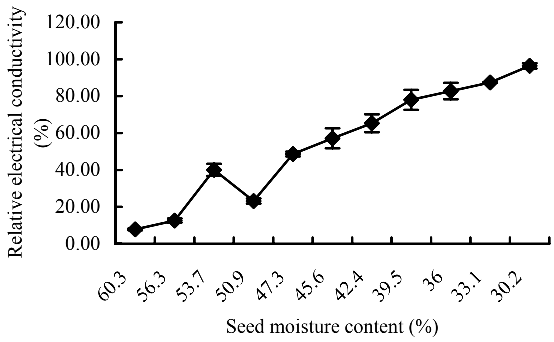

Fig. 3 Effect of air drying on electrical conductivity of *Aesculus chinensis* seeds

Effect of desiccation on activity of SOD in seeds

As shown in Fig. 4, the SOD activity decreased rapidly with daily desiccation except for an abnormality when seed moisture content was in range of 53.7%–50.9%. The phenomenon occurred in the same moisture content range that showed anomalous results for relative electrical conductivity.

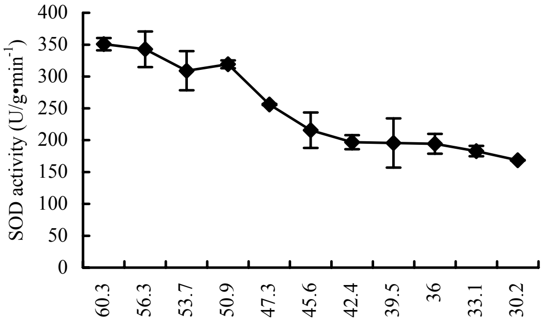

Fig. 4 Effect of air drying on SOD activity of *Aesculus chinensis* seeds

Effect of desiccation on MDA content in seeds

The MDA content increased slowly at the early stages of desiccation and increased more rapidly after the moisture content had been reduced to less than 50.9% (Fig. 5). The only abnormality was at the point where the moisture content was 53.7%. One reason might be that the cell membrane was leakier at the beginning of desiccation.

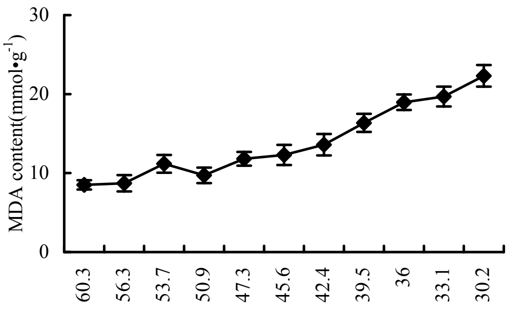

Fig. 5 Effect of air drying on MDA content of *Aesculus chinensis* seeds

Effect of desiccation on soluble sugar content in seeds

The soluble sugar content in seeds slowly increased with daily desiccation. This may be a stress response (Fig. 6).

Discussion

The moisture content of *A. chinensis* seeds was 60.3% at the time of maturation. It reduced gradually when air-dried. The germination percentage increased slightly in the early days of desiccation and then decreased rapidly. This response is similar to seeds of *Eriobotrya japonica* (Chen *et al*. 1998). The germination percentage was zero after 30 days of desiccation when seed moisture content had decreased to 30.2%, indicating that *A. chinensis* seeds are very sensitive to desiccation.

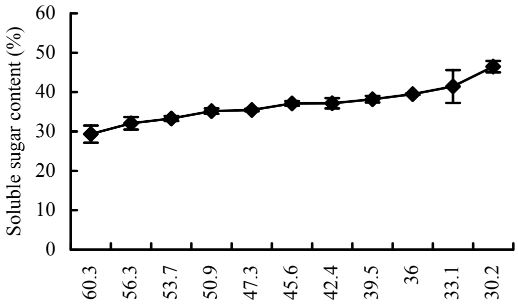

Fig. 6 Effect of air drying on soluble sugar content of *Aesculus chinensis* seeds

The integrity of cell membranes is the basis of seed vigor, and the damage of cell membranes is a sign of seed ageing. It was found that the electrical conductivity of silver maple seeds increased during desiccation (Becwar *et al*. 1982). The present study showed that cell membranes in recalcitrant seeds could be damaged during desiccation. This study also found that there is a possible positive relationship between seed vigor and electrical conductivity at the beginning of desiccation when seed moisture content is still high.

There is also an apparent relationship between the generation of free radicals as deducted from the increasing MDA evolution and cell membrane damage during desiccation. SOD is an important enzyme which dismutates the free radical superoxide. This study showed that the SOD activity decreased rapidly with an increase in the period of desiccation, except for an abnormal, a small peak when seed moisture content was in range of 53.7%–50.9%. The overall trend of decreasing SOD activity implies that the accumulation of free radicals will be aggravated with the increased time of desiccation. This could be one of the reasons of the loss of seed viability. This also confirmed the findings of Li and Sun (1999), who suggested that the decline of seed vigor was related to a decrease in the activities of free radical scavenging enzymes such as superoxide dismutase, ascorbate peroxidase and peroxidase.

MDA is a major indicator of lipid peroxidation. Furthermore, the MDA itself is also a poisonous substance in plant cells. Therefore, the determination of MDA content during seed desiccation is significant. It was found in this study that the MDA content increased slowly at the beginning of desiccation and had a rapid reduction when the moisture content was reduced to 50.9%. This means that the lipid peroxidation in seeds could be accelerated when their moisture content was less than 50.9%.

Sugar is a kind of osmo-protentant. The soluble sugar will be accumulated to keep cells from injury during desiccation. Lin and Chen (1995) reported an increase in sucrose concentration when seeds of *Machilus thunbergii* were desiccated and suggested that sucrose had the function of osmo-conditioning. However, Li and Sun (1999) proposed that desiccation sensitivity of cocoa axes was not associated with sugar-related protective mechanisms. Our study showed that the soluble sugar content in seeds slowly increased with the increasing period of desiccation. It expresses that *A. chinensis* seeds have a kind of mechanism of osmoregulatory.

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